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## Cell Differentiation Induced by Poly(ADP-ribose) Synthetase Inhibitors

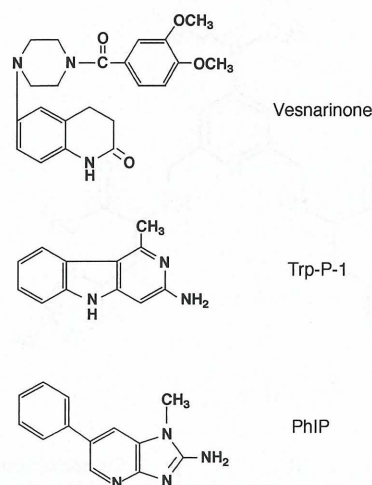
Kunihiro Ueda, Marek Banasik, Sachiko Nakajima, Hye-Young Yook and Takahiro Kido

We recently found two groups of inhibitors of poly(ADP-ribose) synthetase, *i.e.* vesnarinones and heterocyclic amines, to induce differentiation of murine teratocarcinoma EC cells in culture. Cell morphology changed almost completely in 5 to 7 days after treatment with 70  $\mu$ M benzylvesnarinone or 1 mM PhIP. Analyses of poly(ADP-ribose) synthesis and NAD concentrations in EC cells suggested that poly(ADP-ribose) might play a role in initiation of cell differentiation.

**Keywords:** Teratocarcinoma / NAD / Vesnarinone / Heterocyclic amines / Automodification

Poly(ADP-ribose) is a macromolecule synthesized from NAD in nuclei of eucaryotic cells (1). After 30 years from its discovery, precise biological functions of poly(ADP-ribose) remain still unclear. In order to obtain useful tools for *in vivo* studies, we made an extensive survey of poly(ADP-ribose) synthetase inhibitors for years, and found a number of potent inhibitors (2). Recently we added several more to the list of inhibitors, among which were vesnarinones and heterocyclic amines [3]. In this report, we present evidence that these new inhibitors are capable of inducing cell differentiation and that poly(ADP-ribose) plays a role in this cellular event.

Vesnarinone (Fig. 1) is a therapeutic drug used for cardiac failure. Its action is reportedly to increase the intracellular  $\text{Ca}^{2+}$  concentration [4]. We found this compound to inhibit the activity of poly(ADP-ribose) synthetase. The inhibition was not very strong; 14 and 32% inhibition was observed at 0.1 and 0.5 mM, respectively. Other derivatives were less potent.



**Figure 1.** Chemical structures of vesnarinone (3,4-dihydro-6[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1*H*)-quinolinone), Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole), and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine).

### BIOORGANIC CHEMISTRY — Molecular Clinical Chemistry —

#### Scope of research

*This laboratory was founded in 1994, aiming at the linkage between basic sciences and clinical medicine. Thus, our research scope encompasses structures/functions of various biomolecules, their abnormalities causing diseases, and their molecular mechanisms of control useful to therapy. Our current interest is focussed on pathophysiological roles of poly(ADP-ribose) in carcinogenesis and apoptosis, and of A/ $\beta$ -amyloid precursor proteins in Alzheimer's disease. Gene technology and its application to clinical diagnosis are another target of our current effort.*



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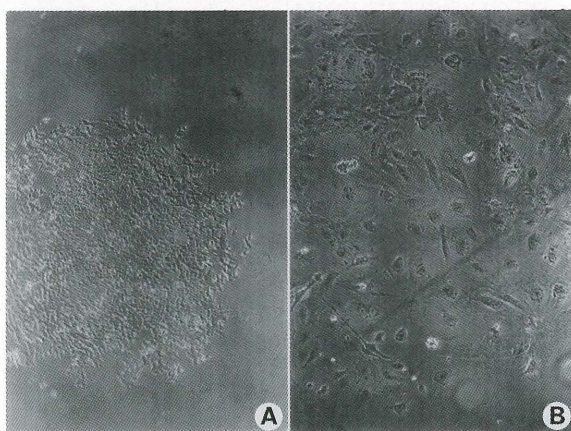


Among heterocyclic amines or pyrolysis products of various amino acids, Trp-P-1 and Trp-P-2 are the most potent in mutagenesis and/or carcinogenesis [5]. We found these  $\gamma$ -carbolines to inhibit the poly(ADP-ribose) synthetase activity. Trp-P-1 was a strong inhibitor ( $IC_{50} = 0.22$  mM), while Trp-P-2 was a weak inhibitor ( $IC_{50} = 2.2$  mM). In addition to Trp-P-1 and Trp-P-2, many other heterocyclic amines, including PhIP that is the most abundant in foods, proved to be, more or less, inhibitory to poly(ADP-ribose) synthetase (6).

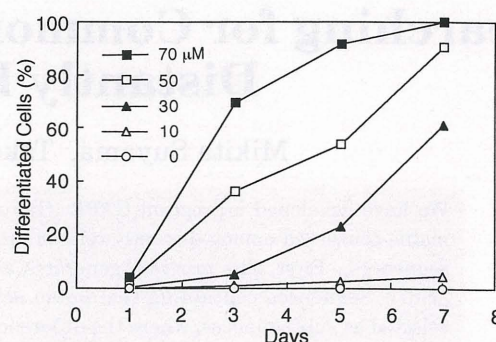
We recently found that vesnarinones and heterocyclic amines are also capable of inducing differentiation of murine teratocarcinoma EC cells. EC cells are pluripotent stem cells and differentiate into many cell types when treated with inducers, including inhibitors of poly(ADP-ribose) synthetase [1, 6].

The EC cells grew in small-cell mass, free of contact inhibition, in control culture. When the cells were cultured for a day or longer in the presence of vesnarinone, benzylvesnarinone, Trp-P-1, or PhIP, the cell mass dispersed, and each cell became larger, flatter and contact-inhibited (Fig. 2). Studies with varying concentrations of benzylvesnarinone (Fig. 3) showed that the cells started to change morphology from the second day, and almost all cells treated with  $>50$   $\mu$ M of the drug acquired the differentiated cell shape in a week.

We then analyzed acceptors and extents of poly(ADP-ribose) synthesis in the course of EC cell differentiation. Our preliminary data indicated that the intracellular NAD concentration decreased precipitously within a day after treatment with an inducer of differentiation (e.g. all-*trans*-retinoic acid), and, at the same time, endogenous poly(ADP-ribose) synthesis on various proteins including the synthetase itself increased remarkably, whereas the synthetase activity, as assayed *in vitro*, continued to decrease throughout the course (6). The transient activation of the synthetase automodification at an early stage of differentiation appeared to be relevant to massive DNA fragmentation and apoptosis induced by the differentiation inducer.



**Figure 2.** Phase-contrast photomicrographs of murine teratocarcinoma EC cells cultured (A) with no addition for 5 days or (B) with 1 mM PhIP for 7 days (6).



**Figure 3.** Time course of EC cell differentiation induced by varying concentrations of benzylvesnarinone (6).

These results, together with our previous observations with other inhibitors of poly(ADP-ribose) synthetase, such as 3-aminobenzamide [7] or 4-hydroxyquinazoline [6], support the view that induction of teratocarcinoma cell differentiation is a property common to inhibitors of this enzyme. Thus, poly(ADP-ribose) appears to participate in cell differentiation at an early stage of induction.

In addition to EC cells, other malignant cells, including Friend erythroleukemia cells and HL-60 promyelocytic leukemia cells, have been shown to be induced to differentiate by poly(ADP-ribose) synthetase inhibitors. These results may suggest a novel way of chemotherapy, that is, normalizing malignant cells by induction of differentiation.

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